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(71) Applicant *(for all designated States except US)*: **OAKWOOD LABORATORIES L.L.C.** [US/US]; 7670 First Place, Oakwood, OH 44146 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **THANOO, Bagavathikanun, Chithambara** [IN/US]; 6584 Glencoe Drive, Brecksville, OH 44141 (US). **MURTAGH, James** [US/US]; 89 Division Street, Hudson, OH 44236 (US).

(74) Agent: **SHARPE, Richard, A.**; Watts, Hoffmann, Fisher & Heinke Co., L.P.A., P.O. Box 99839, Cleveland, OH 44199-0839 (US).

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(54) Title: SLOW RELEASE MICROSPHERES

(57) Abstract: A continuous process for producing microspheres containing an active agent wherein the microspheres have an average cross sectional porosity of less than 5 % of the total cross sectional area. The microspheres are effective for releasing active agent at a constant and highly uniform rate into a surrounding physiological medium. The microspheres comprise a homopolymer of lactic acid or a copolymer of glycolic and lactic acids. An active agent is homogeneously distributed within a matrix of the homopolymer or copolymer wherein an average number and size of the active agent in a particular unit area is substantially the same as a second average number and size of the active agent in a different unit area of the microsphere.

SLOW RELEASE MICROSPHERES

Cross-Reference to Related Application

This application is a Continuation-In-Part application of, and claims priority from, U.S. Patent Application Serial No. 08/800,924 entitled "Continuous Microsphere Process" filed on February 13, 1997, hereby incorporated by reference in its entirety.

Field of Invention

This invention relates to microspheres containing a polypeptide. More particularly, this invention relates to biodegradable microspheres containing LH-RH or its analogs for administration which provide continuous slow release of LH-RH or its analogs into a physiological medium.

Background of the Invention

Microcapsules and Microspheres formed from various natural and synthetic polymers and resins have become popular delivery vehicles for various active agents such as drugs, diagnostic reagents and the like. Degradable microcapsules and microspheres are of particular interest for use in so called "depot" formulations, where delivery of the active agent over an extended period of time is desired.

Sustained release formulations have two generally recognized drug release profiles. The first release profile is characterized by an initial burst of released drug followed by a continuous supply of an effective amount of the drug over an extended period of time. The initial burst is controlled by diffusion of drug located on the external surfaces of a microsphere or microcapsule into a physiological medium upon administration. After the initial diffusion phase, the drug release is continuously released and the rate of release is essentially controlled by degradation of the polymeric binder or matrix material from which the drug containing microsphere or microcapsule is made. Alternatively, the second type of release profile is characterized by not having an initial burst at all. Without an initial burst, the release of drug is essentially controlled by degradation of the polymer binder or matrix.

Depending upon the application it may be desirable to control the release profile to provide microspheres with or without an initial burst followed by sustained continuous and effective release of the drug. For example, leuprolide acetate is a drug wherein an initial burst upon administration may be desirable. Leuprolide acetate is an agonist derivative of Leutenizing Hormone-Releasing Hormone, (LH-RH, also called gonadotropin-releasing hormone) which is known to control and regulate both male and female reproduction. Leuprolide acetate is used primarily as an antineoplastic agent in the treatment of, among others, endometriosis, anemia secondary to leiomyoma, breast neoplasm, prostate neoplasm, endometrial neoplasm, and uterine neoplasm.

In the palliative treatment of prostate neoplasm, leuprolide acetate acts by suppressing testosterone levels. The presence of testosterone is well known to promote the growth of cancerous cells in the prostate. As such, leuprolide acetate offers an alternative to an orchiectomy (surgical removal of the testicles), or estrogen administration. The mechanism for testosterone release is generally known and it has been determined that upon administration of leuprolide acetate there is an initial increase in testosterone levels. The versatile process as disclosed by Applicants allows the user to prepare microspheres containing leuprolide acetate with either an intial burst or no initial burst release profile. It is believed that leuprolide acetate microspheres having an initial burst may combat the increase of testosterone observed upon initial administration and as such, is important for the palliative treatment of prostate cancer. Alternatively, therapeutic sustained release applications having no initial burst release can be made using the same process. This is advantageous for those active agents that do not need an initial burst for their therapeutic treatment. Thus, it is desirable to be able to produce microspheres having any desired amount of initial drug release burst.

A difficulty with obtaining a constant rate of drug release after the initial burst is the size of the drug particles or drug areas within the microsphere. The sizes of the drug particle or area within a microsphere/microcapsule will affect the release of the drug into the surrounding medium. Where large areas or large particles of drug are observed, a concentration gradient exists and it is expected that the drug release will be

higher in those areas as the polymer binder degrades. This can result in secondary or subsequent bursts that are undesirable and difficult to control. Conversely, where the drug particles are very small or finely integrated within the surrounding polymer matrix, the release of drug into the surrounding medium will be much more uniform and regular. To minimize drug release variation it is desirable to have the drug uniformly and finely distributed throughout the polymer matrix so as to provide the most regulated and controlled release of drug into the medium. Thus, it is desirable for the drug to be released at a constant and uniform rate without any spikes or depressions in the release profile.

Another problem with controlling drug release is the degree of porosity of the microsphere. Large pore areas can affect diffusion of the drug into the surrounding medium. If the pore is a void or a space, the surrounding medium will fill these voids or spaces as the polymer degrades. The effect is that channels are formed within the microsphere resulting in an increased amount of drug being released than if the microsphere was degraded primarily externally. Alternatively, and also problematic, is where the pores are filled with drug. As noted above, pores having large areas filled with drug are not desirable for a well controlled and regulated continuous drug release. Thus, it is preferable to provide a microsphere wherein the porosity is minimized and the drug is finely distributed throughout the polymer matrix to provide a highly uniform and continuous release of drug into the surrounding medium. Moreover, it is desirable to have slow release microspheres that can be easily and economically produced.

Summary of the Invention

The present invention is generally directed to continuous release microspheres for releasing effective amounts of a drug into a surrounding physiological medium. The microspheres comprise a polymer of glycolic acid or lactic acid or a copolymer of glycolic and lactic acids. An active agent is homogeneously distributed within a matrix of the polymer or copolymer wherein an average number and size of the active agent in a particular unit area is substantially the same as a second average number and size of the active agent in a different unit area of the microsphere. The active agent is

hereinafter defined as a drug or substance used as a medication or in the preparation of medication. Examples of active agents include steroids, diuretics, carbohydrates, amino acids, proteins, enzymes, peptide hormones, analgesic agents, histamine and antihistaminic agents, cardiovascular agents, local anaesthetic agents, antimalarials, antibiotics, antineoplastics, CNS depressants and stimulants, adrenergic agents, cholinergics, sulfonamides, sulfones, folate reductase inhibitors, vitamins, diagnostic agents, chelating agents and anti-infective agents. Other active agents will be apparent to those skilled in the art in view of this disclosure.

Preferably, the microspheres have an average cross-sectional porosity less than 10% and still, more preferably, less than 5% of the total cross-sectional area. The inventive microspheres release drug in a highly uniform and well controlled rate. The copolymer preferably has an average molecular weight from about 26,000 to about 36,000 and a ratio of glycolide to lactide of about 1:1.

More preferably, the invention is directed to microspheres wherein the active agent is leuprolide. The leuprolide containing microspheres have an average particle size of from about 10 μm to about 40 μm, and an active agent loading of at least about 9%. More preferably, the leuprolide containing microspheres have an active agent load of at least about 15%. It has been found that active agent 15% or greater is effective for providing a leuprolide containing microsphere with a desired initial burst drug release profile.

In another preferred embodiment, the slow continuous release microspheres release drug from the microsphere by essentially polymer degradation in the aqueous physiological medium.

In either embodiment, the drug within the microsphere is continuously released in an effective amount from each said microsphere over a period of about 30 days. Moreover, primarily depending on the polymer composition and choice of active agent, the active agent can be effectively released for longer periods of at least 90 days or again, depending on the polymer composition and choice of active agent for a period of at least 120 days.

Many additional features, advantages and a fuller understanding of the invention will be had from the following detailed description of the preferred

embodiments.

Detailed Description

The present invention is directed to advantageously substantially non porous agent containing polymer bodies, and more particularly to microspheres with excellent homogeneity of drug distribution throughout. The microspheres produced according to the invention are ideal for carrying drugs, diagnostic reagents, or various other active agents. In addition, the microspheres of the present invention can be advantageously produced by a simple, continuous, economic and efficient process which produces a product having uniform characteristics throughout the production cycle.

The term "pore" is hereinafter defined as interstices or areas within the polymer matrix that do not contain polymer or any other inclusion other than the drug or active agent. Thus, the pore can be a void or space or alternatively, a void that has been filled or partially filled with, for instance, a drug retaining substance. Drug retaining substances are those substances as disclosed in, for example, U.S. Patent No. 5,631,021 to Okada et al.

It will be apparent to those of ordinary skill in the art in view of the present disclosure that the active agent can be any agent for which encapsulation or interspersion within a small polymer body is desired. Preferably, the active agent is a drug or substance as defined above and the microspheres are intended for the delivery of such drug or substance to a patient in need thereof. A representative list of suitable drugs and other active agents may be found in U.S. Patent Nos. 5,407,609, 4,767,628, 3,773,919 and 3,755,558, all incorporated herein by reference. Of particular interest are LH-RH agonists such as leuprolide, triptorelin, goserelin, nafarelin, historelin and buserelin, LH-RH antagonists, somatostatin analogs such as octreotide, human, salmon and eel calcitonin, growth hormones, growth hormone releasing hormones, growth hormone releasing peptide, parathyroid hormones and related peptides, interferon, erythropoietin, GM-CSF, G-CSF, thymosin, antitrypsin, enterostatin, and chemotherapy drugs, antibiotics and analgesics for regional administration. An especially preferred drug for use in the instant invention is leuprolide.

In order to incorporate the active agent into the dispersed phase for production of microspheres in accordance with the process of the invention it is usually necessary to dissolve the active agent in a solvent. Solvents for the active agent will of course vary depending upon the nature of the agent. Typical solvents that may be used in the dispersed phase to dissolve the active agent include water, methanol, ethanol, dimethyl sulfoxide (DMSO), dimethyl formamide, dimethyl acetamide, dioxane, tetrahydrofuran (THF), methylene chloride, ethylene chloride, carbon tetrachloride, chloroform, lower alkyl ethers such diethyl ether and methyl ethyl ether, hexane, cyclohexane, benzene, acetone, ethyl acetate, and the like. Selection of suitable solvents for a given system will be within the skill in the art in view of the instant disclosure.

Polymers useful in the present invention can also vary. Examples of polymers known to those of ordinary skill in the art, and useful in the present invention, may be found in, for example, U.S. Patent Nos. 4,818,542, 4,767,628, 3,773,919, 3,755,558 and 5,407,609, incorporated herein by reference. In selecting a particularly desirable polymer for a given system, numerous factors can be considered for purposes of producing a product having the desired clinical characteristics such as biodegradability (e.g., release profile) and biocompatibility. Once one of ordinary skill in the art has selected a group of polymers that will provide the desired clinical characteristics, then the polymers can be evaluated for desirable characteristics that will optimize the manufacturing process. For example, in some instances, it may be possible to select a polymer that will interact with the active agent in a manner that will facilitate the processing of the microspheres, enhance drug load, enhance solvent removal from the dispersed phase or inhibit drug migration from the dispersed phase into the continuous phase.

One consideration in selecting a preferred polymer is the hydrophilicity/ hydrophobicity of the polymer. Both polymers and active agents may be hydrophobic or hydrophilic. Where possible it is desirable to select a hydrophilic polymer for use with a hydrophilic active agent, and a hydrophobic polymer for use with a hydrophobic active agent. In the preferred LH-RH microspheres, an ionic interaction between the drug and the hydrophilic carboxyl groups of the polymer is believed to enhance the drug load. In general, however, since hydrophilic drugs are soluble in water, if there is

no affinity between the polymer and drug, or solidification is not sufficiently fast, drug load may decrease. It is also possible to use a hydrophilic drug in a hydrophobic polymer.

In selecting a particular polymer, the effect of the hydrophobicity/hydrophilicity of the polymer on the residual solvent in the system should also be considered. A hydrophilic polymer can be expected to yield low residual solvent with a hydrophilic drug, such as a hydrophilic peptide. In the case of the preferred leuprolide microspheres, the drug has a tendency to help eliminate hydrophobic solvent from the dispersed phase droplets quickly and efficiently. In addition, it has been observed that a greater drug load tends to correlate to lower residual solvent concentrations. Thus, in some systems, there is an indirect benefit with lower residual solvent when incorporating hydrophilic drugs in hydrophilic polymers. However, since there are other influencing factors on residual solvent other than hydrophilicity, this effect may not uniformly apply to non-peptide drugs. Nevertheless, it should follow that active agents that enhance the elimination of solvent from the dispersed phase droplet, without concomitant drug loss, yield superior products.

Another consideration is molecular weight of the polymer. While the molecular weight of the polymers will obviously impact on the product characteristics such as release rate, release profile and the like, it can also impact the process of producing the microspheres. Higher molecular weight polymers are typically associated with a more viscous dispersed phase, resulting in larger particles or increased difficulties in obtaining small particles and, in some instances, increased residual solvent. By contrast, lower molecular weight polymers are typically associated with slower solidification because the polymer tends to be more soluble. In the preferred system, higher drug loading and enhanced incorporation efficiency has been found to result from the use of higher molecular weight polymers. One advantage of the inventive process is its ability to form well formed, essentially spherical, low residual solvent microspheres with high molecular weight polymers and, hence, viscous dispersed phases. Of course, the particular selection will also depend upon the desired product characteristics. For example, the higher the molecular weight, the longer the degradation time in the body and the longer the duration of drug release.

Still further, the particular polymer concentration employed can effect the system, not only from a product morphology standpoint, but also from a processing standpoint. An increase in polymer concentration tends to be associated with a higher drug load because a viscous dispersed phase needs to eliminate less solvent for solidification. An increased solidification rate tends to cause higher drug retention. Moreover, a viscous dispersed phase leads to less drug diffusion into the continuous phase during solidification. In some systems this may also result in higher residual solvent. In the preferred embodiments, polymer concentration in the dispersed phase will be from about 5 to about 40%, and still more preferably from about 8 to about 30%.

Especially preferred polymers are homopolymers of lactic acid, or copolymers of lactic acid and glycolic acid, i.e., poly(lactide-co-glycolide) or "PLGA" polymers. The ratio of lactic acid residues to glycolic acid residues can vary, and will typically range from 25:75 to 75:25, although even a 10% glycolide could find use since high lactide content results in lower viscosity and higher solubility. Preferred copolymers for 30 day formulations comprise at least about 50% lactic acid residues, such as 50:50 or 75:25 polymers. Preferred copolymers for 90 or 120 day formulations comprise a higher amount of the lactide monomer to glycolide monomer. More preferably, the 90 or 120 day formulations comprise a homopolymer of polylactide. Poly(lactide-co-glycolide) copolymers and polylactide homopolymers are commercially available from a number of sources and can be readily prepared by conventional synthetic routes. Boehringer Inglehiem produces suitable polymers under the designations RG 502, RG 502H, RG 503, RG 503H, RG 752, RG 756, R202H and others. With the preferred LH-RH microspheres RG502H and RG503H are used in the dispersed phase in concentrations of 23% and 14% respectively. Such copolymers also may be made by polymerizing lactic acid and glycolic acid or, preferably, by polymerizing the cyclic dimers of lactic acid and glycolic acid, namely lactide and glycolide, as described in, for example, U.S. patent number 3,773,919, incorporated by reference above. Selection of a suitable polymer for a given system would be apparent to those of ordinary skill in the art in view of this disclosure.

Solvents for the polymer will also vary depending upon a number of factors,

including the nature of the polymer, the active agent, toxicity, compatibility with other solvents in the system and even the use to which the microsphere will be put. Thus, in addition to dissolving the polymer, the solvent must be immiscible with the continuous phase in order to form droplets, highly volatile for optimum evaporation efficiency, and desirably non-flammable for safety reasons. Solvents suitable for the preferred poly(lactic) or poly(lactide-co-glycolide) polymers include methylene chloride, chloroform, ethyl acetate, substituted pyrrolidone and the like. In some instances, the solvent for the active agent will be the same as the solvent for the polymer. Some drugs, typically diagnostic agents such as radioactive inorganic salts used in imaging analysis, are not soluble or only slightly soluble in organic solvents. In these instances, a fine, sub-sub micron size powder can be directly suspended in the polymer solution to form microspheres. Although resort to this will be rare in drug delivery, it may prove useful with diagnostic agents. Selection of other solvents useful in accordance with the process of the invention will be within the skill in the art in view of the instant disclosure.

The polymer, active agent and solvent or solvents are combined to form the dispersed phase. In the preferred embodiment, the dispersed phase is a true, homogeneous solution which may be prepared by mixing the polymer, solvent and active agent together to form a solution. Alternatively, separate solutions of polymer and active agent can be prepared, each in its own solvent, and subsequently mixed to form the dispersed phase solution. In some instances, due to the nature of the active agent and/or polymer, the dispersed phase must be formed as an emulsion. For example, when a given proteinaceous drug is dissolved in a suitable active agent solvent, the resulting solution may be completely immiscible with a solution of the polymer in a particular polymer solvent. In order to provide a relatively homogeneous dispersed phase in which the drug and polymer are relatively uniformly interspersed, the drug and drug solvent may be emulsified with the polymer and polymer solvent to form a dispersed phase emulsion. Upon introduction of the dispersed phase into the continuous phase a w/o/w emulsion is formed. In still other systems, the dispersed phase can be prepared by forming a direct suspension of the active agent in a polymer solution.

In accordance with the inventive process described below, the dispersed phase heretofore described is dispersed or emulsified in a continuous phase in order to form droplets or inclusions of dispersed phase in the continuous phase. As used herein the terms emulsified or dispersed are intended in their broadest sense as meaning discrete regions of dispersed phase interspersed within the continuous phase. The noted inclusions will typically occur as generally spherical droplets, but may in some instances be irregular inclusions due to particular emulsification conditions. Any suitable medium in which the dispersed phase will form droplets or inclusions may be used as a continuous phase, with those that provide a maximum solvent sink for the dispersed phase solvent being especially desirable. Frequently, the continuous phase will also contain surfactant, stabilizers, salts or other additives that modify or effect the emulsification process. Typical surfactants include sodium dodecyl sulphate, dioctyl sodium sulfo succinate, span, polysorbate 80, tween 80, pluronics and the like. Particular stabilizers include talc, PVA and colloidal magnesium hydroxide. Viscosity boosters include polyacrylamide, carboxymethyl cellulose, hydroxymethyl cellulose, methyl cellulose and the like. Buffer salts can be used as drug stabilizers and even common salt can be used to help prevent migration of the active agent into the continuous phase. One problem associated with salt saturation of the continuous phase is that PVA and other stabilizers may have a tendency to precipitate as solids from the continuous phase. In such instances a particulate stabilizer might be used. Suitable salts, such as sodium chloride, sodium sulfate and the like, and other additives would be apparent to those of ordinary skill in the art in view of the instant disclosure.

In the preferred embodiment, the continuous phase is water. The aqueous continuous phase will typically include a stabilizer. A preferred stabilizer is polyvinyl alcohol (PVA) in an amount of from about 0.1% to about 5.0%. Still more preferably, PVA is present in an amount of about 0.35%. Other stabilizers suitable for use in the continuous phase would be apparent to those of ordinary skill in the art in view of the instant disclosure.

The selection of particular polymers, solvents and continuous phases will of course vary depending on the active agent and the desired product characteristics. Once the desired product characteristics, such as clinical application, release profile

and the like are established, there may nevertheless be some latitude in selecting polymers, solvents and continuous phases to facilitate the production process.

For example, in slow solidifying systems, or systems where small particles are desired, a viscous continuous phase and a higher concentration of stabilizer may be necessary to obtain the desired microspheres. Likewise, if necessary, the dispersed phase can be made more viscous by cooling, increasing the molecular weight of the polymer or increasing the concentration of the polymer. Of course, adjusting the viscosity of the continuous phase further complicates the process, and use of a dispersed phase with a high viscosity makes it more difficult to obtain small particles. Still further, in addition to complicating both the process and apparatus, cooling the dispersed phase will have a tendency to reduce the solubility of the dispersed phase solvent therein, which can lead to higher residual solvent contents and/or longer solvent removal periods. Drug crystallization might also be a problem with cooling. An advantage of the preferred embodiment of the invention is that, because foaming is not a significant impediment, it is not necessary to cool or otherwise adjust the viscosity of the phases in order to obtain small particle sizes. The present process enables one to obtain small particle sizes even when it is necessary to use a viscous dispersed phase, without having to adjust the viscosity of the continuous phase to prevent foaming. This simplifies the process and reduces costs.

Advantageously, once the desired drug load is obtained, and the parameters of feed rate, temperature, etc. are determined, scaling up to larger batches, including production level batches, becomes a simple matter of running the process longer. No additional feed tubes, emulsifiers, impellers or the like are necessary to produce larger number of microspheres having the desired characteristics. Moreover, the low porosity microspheres produced during the continuous process of the invention are exceptionally uniform in terms of size, agent load and the like, regardless of when during the process they were produced. Still further, the drug release profile during polymer degradation is constant and highly uniform as a result of the low porosity and finely distributed active agent within the microspheres.

These and other aspects of the invention will be further understood from the following non-limiting examples.

Unless otherwise noted the following apparatus was used in the examples. The Silverson in-line mixer was modified with the additional inlet for the dispersed phase as hereinbefore described, and connected to a Silverson stirrer model 4LRT. The outlet tube was connected to a 7 liter jacketed bio-reactor from Applikon. One of the top plate ports of the Applikon was connected to the vacuum pump, another to a dry 0.2 μm filter to serve as the air inlet, another served as the inlet from the Silverson, and the fourth served as the harvest line.

Example 1

This is an example of a typical procedure used to prepare microspheres of poly(lactide-co-glycolide) and Leuprolide (LH-RH agonist derivative).

The hydrophilic polymer RG503H is a 50:50 copolymer of poly(lactide-co-glycolide) from Boehringer Ingelheim having an inherent viscosity of 0.42 dL/g. This polymer has a weight average molecular weight (M_W) on the order of 30,000. A solution of this polymer was prepared by dissolving 7.0 grams RG503H in 36 g dichloromethane. The drug solution was prepared separately by dissolving 1.00 g Leuprolide acetate in 8.56 g methanol. The dispersed phase (DP) was prepared by combining the Leuprolide solution and the polymer solution with mixing. The DP thus formed is a homogeneous light yellow, relatively clear solution. The DP was then transferred into a 124 mL pressure addition funnel and connected to the DP inlet to of the Silverson unit through a micrometer Teflon needle valve. Head pressure was applied to the addition funnel (10 psi) above the continuous phase (CP). The stop-cock of the addition funnel was kept closed until the DP addition started.

The continuous phase (CP) was 0.35% polyvinyl alcohol (PVA) solution (w/v) prepared in a 7 liter beaker by dissolving 14.0 g PVA (cold water soluble, MW 30,000-70,000) in 4000 mL water. The CP addition tube to the Silverson unit from the CP tank used a peristaltic pump for flow control. The outlet tube of the Silverson unit was connected to the solvent evaporation tank, which is a 7 liter Applikon reactor with a jacketed vessel and lip-seal stirrer assembly.

The Silverson unit was primed with CP and trapped air in the cell was removed by opening the bleed valve. The stirrer motor of the Silverson was turned on to 7000

rpm and the CP and the DP were introduced into the reactor simultaneously. The required flow rate of CP and DP were achieved and maintained constant using the peristaltic pump (for CP) and needle valve (for DP). The addition time was 2 minutes, during which 52.6 grams of DP and 4000 mL of CP were introduced to the mixer at a constant rate of flow.

The microspheres were formed in the Silverson unit and delivered as a suspension into the solvent evaporation tank. The head space air was constantly replaced using the vacuum pump. The air flow through the head space was approximately 29 standard liters per minute. The temperature of the evaporation tank was increased from 25° to 42°C and maintained for 3 hours. The higher temperature and air sweep helped the system to achieve lower residual solvent in the microspheres.

After solvent evaporation, the system temperature was lowered to 25°C and the microspheres harvested by pressure filtration (5-20 psi) onto a 5 μm filter using a 2000 mL stirred cell assembly (M-2000 from Amicon). The microspheres were washed with 2000 mL WFI and freeze dried in bulk as a concentrated suspension in WFI (approx. 0.3 g/mL). Of course, this procedure will change upon scale up to commercial production.

The microspheres prepared according to this example had 9.88% drug load showing 79% drug incorporation efficiency. Microscope analysis showed that the microspheres were spherical and the particles ranged from non-porous to partially porous. Small particles were non-porous while larger particles showed some porosity. The bulk density of the microspheres was 0.588 g/cc. The particle size distribution analysis showed that 50% of the particles were below 18 μm (volume distribution), and 80% of the particles were between 7 and 36 μm. The residual solvent (methylene chloride or methanol) was undetectable (i.e., less than about 20 ppm).

Example 2

As illustrated by this example, a significant advantage of the preferred continuous flow process according to the invention is the product consistency during processing. Prior processes are unable to produce microspheres having virtually identical characteristics at the end of the production run as the ones produced at the

beginning and middle of the run. This is a significant commercial advantage.

The microspheres were prepared in the same manner as in Example 1, using 25% excess DP and CP. The DP contained 8.75 g RG503H, 1.25 g Leuprolide acetate, 45 g methylene chloride and 10.7 g methanol. The CP was 5000 mL 0.35% PVA. In this example, the microsphere suspension produced in the Silverson reactor was not transferred to the solvent removal tank. Instead, each 1000 mL fraction (the collection time for each fraction being approx. 24 seconds) were collected in a 2000 mL beaker. Thus, five fractions of equal volume was collected. The microspheres from each fraction were separated by filtration, freeze dried in bulk and compared.

Microscopic analysis showed that the morphology of the microspheres obtained in all five fractions was identical. Larger particles showed some porosity while the smaller particles were non-porous. The following Table I shows that each fraction (Frxn) of microspheres produced throughout the process have excellent consistency.

Table I

	Frxn 1	Frxn 2	Frxn 3	Frxn 4	Frxn 5
Load (%)	11.17	11.31	10.96	11.05	10.99
10% under (μm)	9.6	8.9	8.9	9.3	8.9
50% under (μm)	18.1	17.4	17.8	17.8	17.4
90% under (μm)	33.3	32.6	35.5	34.4	32.6
Bulk Density	0.40	0.48	0.48	0.47	0.48

The residual methylene chloride values were higher in all the fractions (approx. 8000 ppm) because no solvent evaporation was performed on the microspheres.

Example 3

In this example, a hydrophobic polymer was used. Boehringer Ingelheim RG502 is a 50:50 co-polymer of PLGA with an inherent viscosity of 0.2 dl/g. The preparation procedure was similar to Example 1, except for the composition of the DP.

Here, a polymer solution was prepared by dissolving 8.77 g RG502 in 20 g dichloromethane. The drug solution was separately prepared by dissolving 1.25 g leuprolide in 4 g methanol. The polymer and drug solutions were mixed to form the DP. Thereafter, 5000 mL of CP was added by adjusting the micrometer needle valve setting for the DP addition so that the time for both DP and CP addition was approximately the same (2 minutes). Silverson stirring, solvent evaporation and microsphere harvesting were all performed as in Example 1.

The drug incorporation efficiency of the resulting microspheres was 65% and the microspheres had a drug load of 8.17% Microscope analysis showed that the microspheres had spherical geometry and were porous. The bulk density of the microspheres was 0.23. The particle size distribution analysis showed that 50% of the particles were below 25.6 μm (volume distribution), 80% of the particles were between 12.2 and 44.0 μm. The residual methylene chloride and methanol in the microspheres was undetectable (less than 20 ppm).

Example 4

In this example, a homopolymer of polylactic acid was used. 8.75 g polylactic acid (R202H from Boehringer Ingelheim) having an inherent viscosity of 0.18 dL/g was dissolved in 20 g dichloromethane. Drug solution was prepared by dissolving 1.25 g leuprolide in 4 g methanol. The polymer and the drug solutions were mixed to form the DP, which appeared as a homogeneous, nearly colorless solution. The microspheres were prepared and harvested as disclosed in Example 1 using 5000 mL of continuous phase.

These microspheres had a drug incorporation efficiency of 85% and a drug load of 10.58%. Microscope analysis showed that the microspheres had perfect spherical geometry, with most of the spheres appearing non-porous. A few of the larger particles appeared to have pores at the center of the core. The bulk density of the microspheres was 0.615 g/mL. The particle size analysis showed that 50% of the particles were below 16.0 μm (volume distribution), and 80% of the particles were between 5.8 and 30.2 μm. The microspheres contained 79 ppm methylene chloride

and an undetectable amount (less than 10 ppm) of methanol.

Example 5

In this example, the microspheres were prepared as in Example 1 using 8.75 g RG503H, 1.25 g leuprolide, 45 g methylene chloride and 10.7 g methanol for the DP. The stirring speed was increased to 9000 rpm, using 5000 mL CP of 0.35% PVA solution. The drug incorporation efficiency was 70.7% and the drug load was 8.84% in the microspheres. Microscope analysis showed that the microspheres were smaller, had a spherical geometry, and were predominantly non-porous. The bulk density of the microspheres was 0.510 g/mL. The particle size distribution analysis showed that 50% of the particles fall below 15.5 μm (volume distribution) and 80% of the particles were between 8.1 and 24.8 μm. The microspheres contained 47 ppm residual methylene chloride and an undetectable amount of methanol (less than 10 ppm).

Example 6

In this example, microspheres were prepared containing a proteinaceous agent. The active agent was the protein Human Serum Albumin. The microspheres were prepared by forming a w/o/w emulsion using RG503H polymer. The preparation procedure was the same as in Example 1 except that the dispersed phase was formed by preparing a polymer solution of 8.75 g polymer in 45 g methylene chloride. 5 mL of 25% w/v solution of human serum albumin was added slowly into polymer solution while stirring using a magnetic stirrer. The dispersed phase thus obtained was stirred vigorously for about 5 minutes to form a milky white fine suspension. The microspheres were prepared as in Example 1 except that the stirring speed of the Silverson unit was 6000 rpm. The microspheres were harvested and freeze dried as in Example 1.

Microscope analysis showed that the microspheres had perfectly spherical geometry and were highly porous. The bulk density of the microspheres was 0.03 g/mL. The particle size distribution analysis showed that 50% of the particles were below 48.4 μm, and 80% were between 23.0 and 69.7 μm. The microspheres did not

have any detectible residual methylene chloride.

Example 7

In this example, microspheres were prepared from RG503H and a non-peptide drug. The polymer solution was prepared by dissolving 8.74 g RG503H in 45 g dichloromethane. 1.25 g dipyridamole was added slowly to the polymer solution and 2.53 g methanol was added to make the homogeneous solution, which appeared bright yellow. 5000 mL of 0.35% PVA solution was used as the continuous phase. The microspheres were prepared, harvested and freeze dried as in Example 1.

These microspheres had an 88% drug incorporation efficiency with an 11.0% drug load. Microscope analysis showed that the microspheres were spherical, generally smaller and predominantly non-porous. The bulk density of the microspheres was 0.45 g/mL. The particle size distribution analysis showed that 50% of the particles were below 13.5 μm (volume distribution), and 80% of the particles were between 5.8 and 20.0 μm. The microspheres had 107 ppm residual methylene chloride and undetectable methanol.

Example 8

As illustrated by this example, a significant advantage of the preferred continuous flow process according to the invention is the homogeneity of the drug distributed throughout the polymer matrix and the low porosity obtained for each microsphere. The microspheres were prepared according to the process of Example 1 using 9.20 g RG503H, 2.16 g Leuprolide, 41.4 g methylene chloride and 12.0 g methanol for the DP. The stirring speed was 7000 rpm using 4000 mL CP of 0.35% PVA solution. The drug incorporation efficiency was 73.7% and the drug load was 16.12% in the microspheres. The microspheres demonstrated a release profile with an initial burst. The average particle size was about 17.2 microns. The microspheres were prepared, harvested and freeze dried as in Example 1.

Microscopic analysis showed that the morphology of the microspheres obtained

had uniform distribution of the drug and were predominantly non-porous. The microspheres were cross-sectioned to a thickness of about 60nm and photomicrographed by transmission electron microscopy (TEM). The pores were physically counted from at least three different randomly selected microspheres and averaged. The percent area of the pores within the microsphere were measured by placing a transparent graph sheet over the TEM micrographs. The area occupied by each pore within the graphic boundary was measured and added together. The percent area of the pores were determined from a ratio of the total pore area to the microsphere cross sectional area within the graphical boundary to give a numerical value for cross sectional porosity. Further evaluation revealed that the pores were bubbles or voids within the microspheres. It is believed that the pores result from the rapid removal of solvent.

It was also observed that the average number and size of polypeptide particles in a particular unit area of the microspheres are substantially the same as a second average number and size of particles in a different unit area of the microspheres. The unit areas measured were of the same dimension.

The leuprolide active agent within the microsphere were found to be homogeneously distributed within the microspheres. The microspheres were treated with a rabbit antibody double stain specific for leuprolide and labeled with gold. The technique is readily known to those skilled in the art and is capable of detecting a single molecule of leuprolide. TEM analysis showed that the leuprolide labelled with gold were homogeneously distributed throughout each microsphere and that the average number and size of the polypeptide particles were uniform in size.

The following Table II shows the average number of pores per microsphere and % pore area after cross sectional analysis.

Table II

Sample	No. Of Pores	Area of Pores (%)
1	26	2.93

2	27	7.31
3	20	2.79

Comparative Example 1

In this comparative example, leuprolide acetate microcapsules sold under the trade name LUPRON 7.5 were analyzed by the methods disclosed in Example 8. The average microcapsule size was about 20 um. The following Table III shows the results obtained for microcapsules produced by this process.

Table III

Sample	No. Of Pores	Area of Pores (%)
1	167	16.28
2	176	19.17
3	127	17.08

The microspheres were treated with a rabbit antibody double stain specific for leuprolide and labelled with gold. TEM analysis showed that no specific leuprolide staining was observed. This suggests that the leuprolide in these microcapsules were located in the pores and diffused or dissolved in the sectioning medium prior to staining. The pores were found to be irregular and nonuniformly distributed within the microcapsules.

The foregoing description of the preferred embodiments of the invention have been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Obvious modifications or variations are possible in light of the above teachings. The embodiments were chosen and described to provide the best illustration of the principles of the invention and its practical applications to thereby enable one of ordinary skill in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use

contemplated. All such modifications and variations are within the scope of the invention as determined by the appended claims when interpreted in accordance with the breadth to which they are fairly, legally and equitably entitled.

Claims

What is claimed is:

1. Microspheres for continuous release of effective amounts of an active agent in an physiological medium, said microspheres comprising:

a copolymer of glycolic acid and lactic acid;

an active agent wherein said active agent is homogeneously distributed within a matrix of said polymer body wherein an average number and size of the active agent in a particular unit area is substantially the same as a second average number and size of the active agent in a different unit area of the microsphere; and

said microspheres having an average cross-sectional porosity less than 10% of the total cross-sectional area.

2. The microspheres of claim 1 wherein said microspheres having an average cross-sectional porosity less than 5% of the total cross-sectional area.

3. The microspheres of claim 1 wherein said copolymer has a ratio of glycolide to lactide of about 1:1.

4. The microspheres of claim 3 wherein said copolymer has an average molecular weight from about 26,000 to about 36,000.

5. The microspheres of claim 1 wherein said active agent is water soluble.

6. The microspheres of claim 1 wherein said active agent is leuprolide.

7. The microspheres of claim 1 wherein said microspheres have an average particle size of from about 10 μm to about 40 μm, and an active agent load of at least about 9%.

8. The microspheres of claim 1 wherein said microspheres have an average particle size of from about 10 μm to about 40 μm, and an active agent load of at least about 15%.

9. The microspheres of claim 1 wherein the continuous release of said active agent within said polymer matrix is essentially by polymer degradation.

10. The microspheres of claim 1 wherein said active agent is continuously released in an effective amount from each said microsphere over a period of at least about 30 days.

11. The microspheres of claim 1 wherein said active agent is a polypeptide of the following formula:

(Pyr) Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-$NHCH_2CH_3$

or an acetate salt thereof.

12. Microspheres for continuous release of effective amounts of an active agent in an physiological medium, said microspheres comprising:

a homopolymer of lactic acid;

an active agent wherein said active agent is homogeneously distributed within a matrix of said polymer body wherein an average number and size of the active agent in a

particular unit area is substantially the same as a second average number and size of the active agent in a different unit area of the microsphere; and

said microspheres having an average cross-sectional porosity less than 10% of the total cross-sectional area.

13. The microspheres of claim 12 wherein said microspheres having an average cross-sectional porosity less than 5% of the total cross-sectional area.

14. The microspheres of claim 12 wherein said active agent is leuprolide.

15. The microspheres of claim 14 wherein said leuprolide is continuously released in an effective amount from each said microsphere over a period of at least about 90days.

16. The microspheres of claim 14 wherein said leuprolide is continuously released in an effective amount from each said microsphere over a period of at least about 120 days.

17. The microspheres of claim 16 wherein said microspheres have an average particle size of from about 10 μm to about 40 μm, and an active agent load of at least about 15%.

18. A pharmaceutical composition comprising microspheres for slow continuous release of effective amounts of a water soluble active agent in an aqueous physiological medium, said microspheres comprising:

a poly(lactide-co-glycolide) copolymer;

said active agent is a leuprolide drug wherein said leuprolide ranges from about 12 to about 20% and is homogeneously distributed within a matrix of said polymer bodies wherein said leuprolide release is predominantly erosion controlled;

said erosion control degrades the polymeric leuprolide containing matrix and releases a continuous effective amount of said leuprolide into the aqueous physiological medium for at least thirty days; and

each one of said microspheres having a total cross-sectional porosity less than 10% of the total cross-sectional area.

19. The pharmaceutical composition according to claim 18 wherein said poly(lactide-co-glycolide) copolymer are polymer bodies wherein the ratio of glycolide to lactide is about 1 : 1.

20. The pharmaceutical composition according to claim 18 wherein said poly(lactide-co-glycolide) copolymer have an average molecular weight within the range of about and 26,000 to about 36,000.

21. Slow release leuprolide microspheres having a cross-sectional porosity of less than 10% prepared by a process comprising :

a) forming a dispersed phase comprising a homogeneous solution of a leuprolide drug and a copolymer of lactide and glycolide;

b) providing a continuous phase in which said dispersed phase will form an emulsion;

c) continuously introducing dispersed phase into a reactor vessel at a dispersed phase feed rate, and continuous phase into said reactor vessel at a continuous phase feed rate, said reactor vessel including means for forming an emulsion, and forming an emulsion of said dispersed phase in said continuous phase;

d) continuously transporting said emulsion from said reactor vessel to a solvent removal vessel to remove solvent.

22. Slow release leuprolide microspheres having a cross-sectional porosity of less than 10% prepared by a process comprising :

a) forming a dispersed phase comprising a homogenous solution of a leuprolide drug and a homopolymer of lactide;

b) providing a continuous phase in which said dispersed phase will form an emulsion;

c) continuously introducing dispersed phase into a reactor vessel at a dispersed phase feed rate, and continuous phase into said reactor vessel at a continuous phase feed rate, said reactor vessel including means for forming an emulsion, and forming an emulsion of said dispersed phase in said continuous phase;

d) continuously transporting said emulsion from said reactor vessel to a solvent removal vessel to remove solvent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21038

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 9/14, 9/50; C08G 63/08
US CL :424/489, 501; 528/354
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/489, 501; 528/354

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,980,948 A (GOEDEMOED et al) 09 November 1999, see column 1, lines 23-42, column 7, lines 12-40, column 9, lines 37-66, column 15, line 11-12, column 16, line 12 to column 22 and line 53.	1-10 and 12-22
Y	US 5,395,916 A (MOCHIZUKI et al) 07 March 1995, see column 15, line 3 to column 19 and line 63.	1-10 and 12-22
A	US 5,540,937 A (BILLOT et al) 30 July 1996, see entire document.	1-10 and 12-22
A	WO 95/35097 A1 (UNIVERSITY OF NOTTINGHAM) 28 December 1995, see entire document.	1-10 and 12-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 NOVEMBER 2000	Date of mailing of the international search report 02 JAN 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BLESSING FUBARA Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21038

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 11
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 11 was not searched because no computer readable form (CRF) has been filed. The instant invention recites a sequence and cannot be searched other than by sequence search.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.